

University of Groningen

## Electron Microscopy of Ascus Formation in the Yeast *Debaryomyces hansenii*

Kreger-van Rij, N.J.W.; Veenhuis, M.

*Published in:*  
Journal of general microbiology

*DOI:*  
[10.1099/00221287-89-2-256](https://doi.org/10.1099/00221287-89-2-256)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
1975

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Kreger-van Rij, N. J. W., & Veenhuis, M. (1975). Electron Microscopy of Ascus Formation in the Yeast *Debaryomyces hansenii*. *Journal of general microbiology*, 89(2). <https://doi.org/10.1099/00221287-89-2-256>

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

## Electron Microscopy of Ascus Formation in the Yeast *Debaryomyces hansenii*

By N. J. W. KREGER-VAN RIJ AND M. VEENHUIS

*Laboratory of Medical Microbiology and Laboratory of Electron Microscopy,  
Department of Biology, State University, Groningen, The Netherlands*

(Received 17 December 1974; revised 26 January 1975)

### SUMMARY

Ascus formation in *Debaryomyces hansenii* includes fusion of two cells, usually mother and daughter while still attached to each other, through short protuberances developed from the cross wall between them. Nuclear fusion takes place in the channel connecting the two cells; meiosis apparently occurs in the mother cell. Generally, only one lobe of the meiotic nucleus is surrounded by a prospore wall and it becomes the nucleus of a spore with a warty wall. The rest of the nucleus disappears. The spores germinate by swelling in the ascus and forming one or more buds.

### INTRODUCTION

Diploidization by means of conjugation between mother cell and bud occurs in several ascomycetous yeasts. However, there are few descriptions of the mechanism of this conjugation. Guilliermond (1911, 1928) described fusion between an adult cell and its bud when the bud was about to be separated from the mother cell in species of the genera *Zygosaccharomyces* and *Debaryomyces*. Van der Walt (1970) considered the characteristic 'conjugation between mother cell and bud' inappropriate for *Debaryomyces* species, because, according to him, no cellular fusion, only karyogamy, took place. The two authors do not agree on whether a separating wall is present between mother cell and bud before nuclear fusion takes place. Since this question may be answered by electron microscopy, we studied ascus formation of a *Debaryomyces* species in ultrathin sections. Observations on vegetative reproduction and germination of the ascospores were also made.

### METHODS

Strain G592 of *Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij was studied. A sporulating culture was obtained by growing the yeast in a small Petri dish on 1 % glucose-0.3 % yeast extract-0.5 % peptone agar for 2 days at room temperature, and then pouring a thin layer of 0.9 % aqueous NaCl over the culture and leaving it for 1 to 2 days at the same temperature. Germinating spores were obtained by inoculating a sporulating culture on to malt agar or glucose-yeast extract-peptone agar previously covered with cellophane on a slide and culturing them for 24 h at 20 °C.

The harvested cells were washed with water and fixed with one of the following: (i) 1.5 %  $\text{KMnO}_4$  for 15 min at room temperature; (ii) 1.5 %  $\text{KMnO}_4$  for 15 min followed by 1 %  $\text{OsO}_4$  in veronal-acetate buffer pH 7.0 for 1 h, both at room temperature; (iii) 1 %  $\text{OsO}_4$  in veronal-acetate buffer for 16 h at room temperature; (iv) 3 % glutaraldehyde in phosphate buffer (0.1 M, pH 7.2) for 5 h at 4 °C and, after washing with buffer, with 1 %

OsO<sub>4</sub> in veronal-acetate buffer for 16 h at room temperature. All the Figures show material fixed with KMnO<sub>4</sub>. Fixations (ii) (iii) and (iv) were used only for sporulating cultures. After suspending the fixed material in agar, the agar blocks were dehydrated through an ethanol series and post-stained with a saturated solution of uranyl acetate in 100 % ethanol for 1 h. The specimens were embedded in Epon 812; the germinating spores were also embedded in Spurr's (1969) epoxy resin. Some of the sections were post-stained with lead citrate and uranyl acetate. They were examined with Philips EM 300 and EM 201 electron microscopes.

## RESULTS

Cells of *D. hansenii* were spherical or short-ellipsoidal. Buds were usually formed near the two poles, but could also form multilaterally; they sometimes remained attached to the mother cell, forming short branched chains. The size of the bud depended on the conditions of growth.

In sections, the cell wall had a structure typical of ascomycetous yeasts: a thin dark outer layer and a broad light inner layer. The wall of the bud emerged from a cell under the wall of the mother cell, and the wall of the bud within the mother cell was usually very light (Fig. 1). A cross wall between mother and bud was formed centripetally; first a very light primary layer developed and then slightly darker secondary layers were formed on each side of it. In the centre of the cross wall a thin dark line connecting the two cells was observed, the closure line, in which two layers of plasmalemma might be discerned.

Conjugation between mother cell and bud preceded ascus formation. The conjugating buds varied in size, but were usually much smaller than the mother cells. After conjugation, the axis of the bud was frequently oblique rather than perpendicular to the mother cell. Conjugating cells with a short neck between them also occurred. Apart from conjugation between mother cell and bud, conjugation of unrelated cells was occasionally observed; these cells also were connected by a short neck. The ascus contained one, or occasionally two, warty spores.

Before cell fusion, mother cell and bud showed extensions at the edge of bud and birth scars of partly separated cells (Fig. 2). These extensions, which we consider to be outgrowths, fused at the tips and thus formed an open connexion between the cells and a slight bulge at the collar (Figs. 10, 11). Some sections of asci showed that formation of protuberances from the scars of separated cells also occurred (Fig. 4), resulting in a short neck between the cells. Cells connected by protuberances which had not grown out from scars were seldom observed. At the site of fusion, dark bodies within the contacting walls occurred (Fig. 3). Exceptionally, a bud developed from the scar surface and conjugation between the mother cell and this bud took place (Fig. 5). Sections of many asci showed a partly or wholly dissolved cross wall between the conjugating cells, leaving no indication of the formation of outgrowths before fusion. The connexion was usually at the edge of the cross wall, and the closure line was occasionally still visible in the remaining part (Fig. 10).

In fusing cells, one nucleus was observed in the connecting channel between them (Fig. 6) and we assume that this was a diploid nucleus. An early stage of spore formation was visible in the mother cell: one lobe of the nucleus was partly surrounded by a prospore wall consisting of two unit membranes (Fig. 7). At a later stage, wall formation was completed and enclosed a nucleus and other organelles (Fig. 8). A thin dark line was present between the two membranes and, occasionally, round, dark inclusions. A large part of the original nucleus remained in the ascus outside the single spore (Fig. 9) and gradually disappeared. Between the two membranes forming the outer covering of the spore a light layer developed

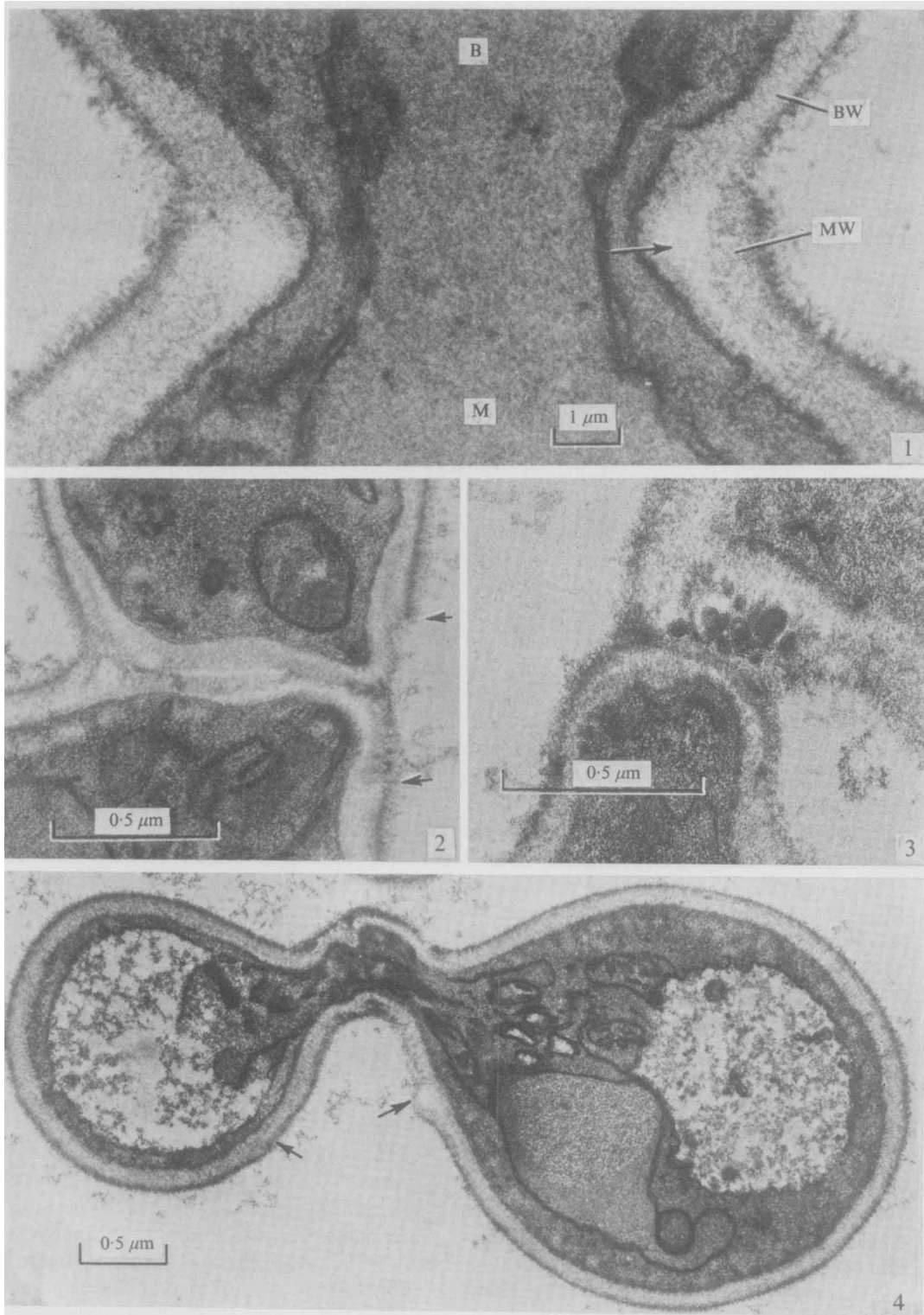


Fig. 1. Section through the collar between mother cell (M) and bud (B). The wall of the bud (BW) emerges from under the wall of the mother cell (MW) and is electron-transparent within the mother cell (arrow).

Fig. 2. Mother cell and bud are separated by a cross wall which has partly split. The edges of bud and birth scars are visible (arrowed). Both cells have formed short outgrowths from the scar surface.

Fig. 3. Site of fusion of two cells with dark bodies in the contacting walls.

Fig. 4. Conjugating cells connected by outgrowths from the scars. The edges of the scars on mother cell and bud are visible (arrows).

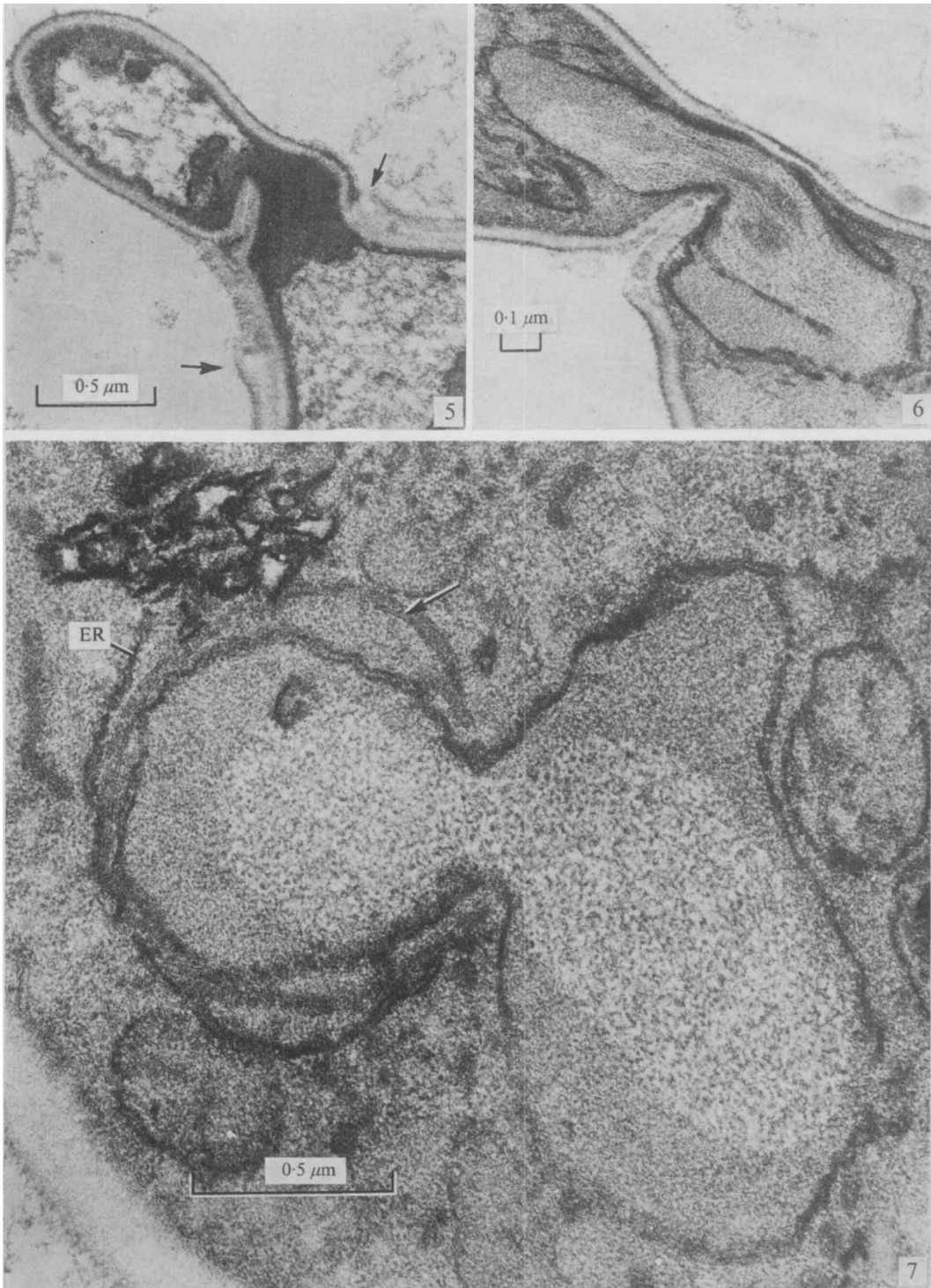


Fig. 5. Part of an ascus resulting from conjugation between a mother cell and a cell budded off from the scar surface left by a previous bud. The scar edges of the first budding are arrowed.

Fig. 6. Nucleus in the channel between two cells after fusion.

Fig. 7. One lobe of the nucleus of fused cells, partly surrounded by the prospore wall (arrowed). Endoplasmic reticulum (ER) lies in the vicinity of this wall.

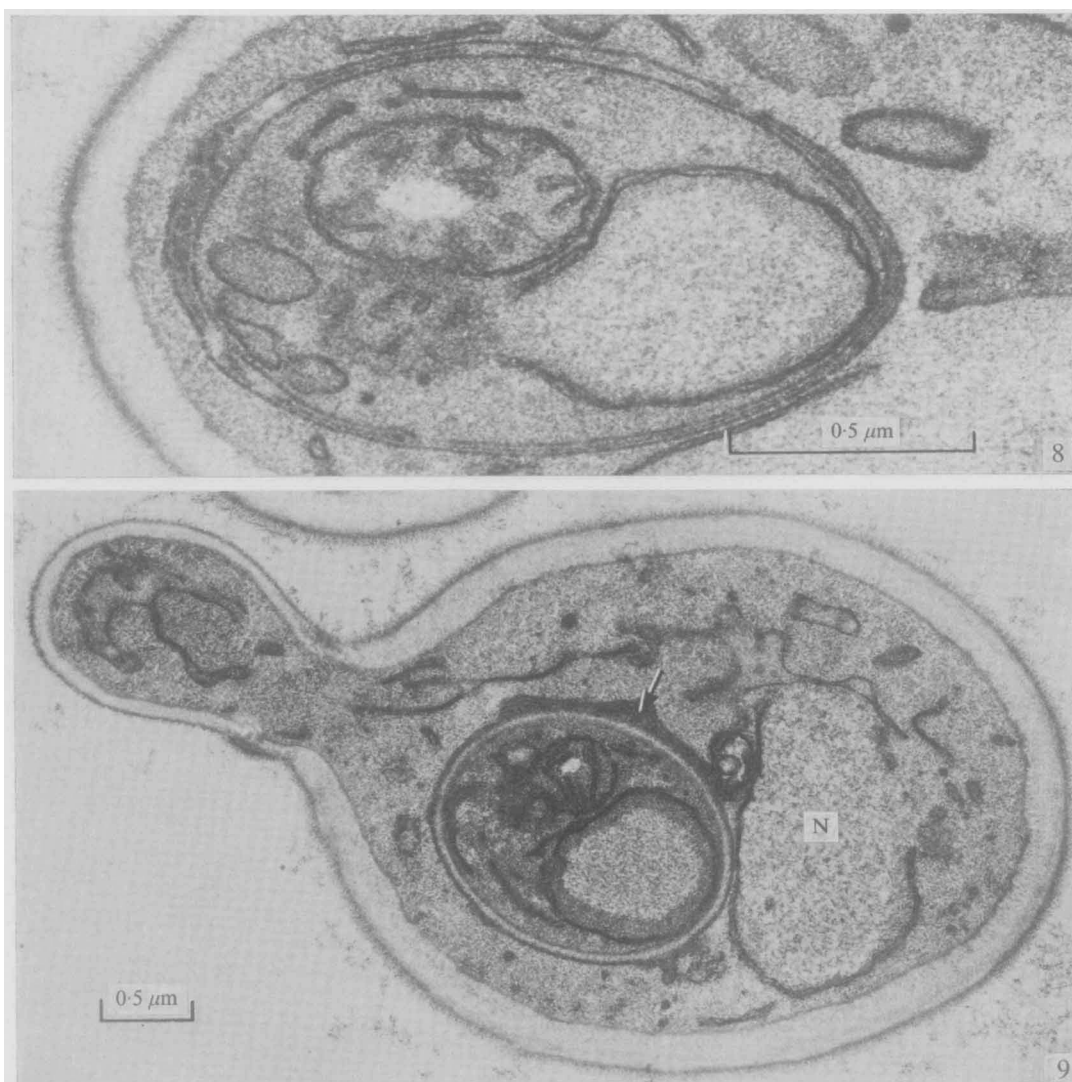


Fig. 8. Very young spore enclosed in the two unit membranes. Between these there is a thin dark line and round, dark inclusions.

Fig. 9. Ascus with a young spore and original nucleus (N) outside it. Membrane structures are present (arrow).

(Fig. 9). The inner membrane became the plasmalemma and the outer membrane the dark outer layer of the spore wall. The wall was initially smooth but became warty at an early stage (Fig. 10). The warts were extensions of the inner layer, but were darker, sometimes with light spots (Fig. 11). Lipid bodies were present both before and during conjugation. They surrounded the young spore, and in  $\text{KMnO}_4$ -fixed cells they took on a reticulate appearance. With all fixations, membranous structures were observed around the spores and elsewhere in the ascus (Fig. 9). Lipid material still adhered to the mature spore. The plasmalemma of the ascus could become detached from the wall and lie close to the spore.

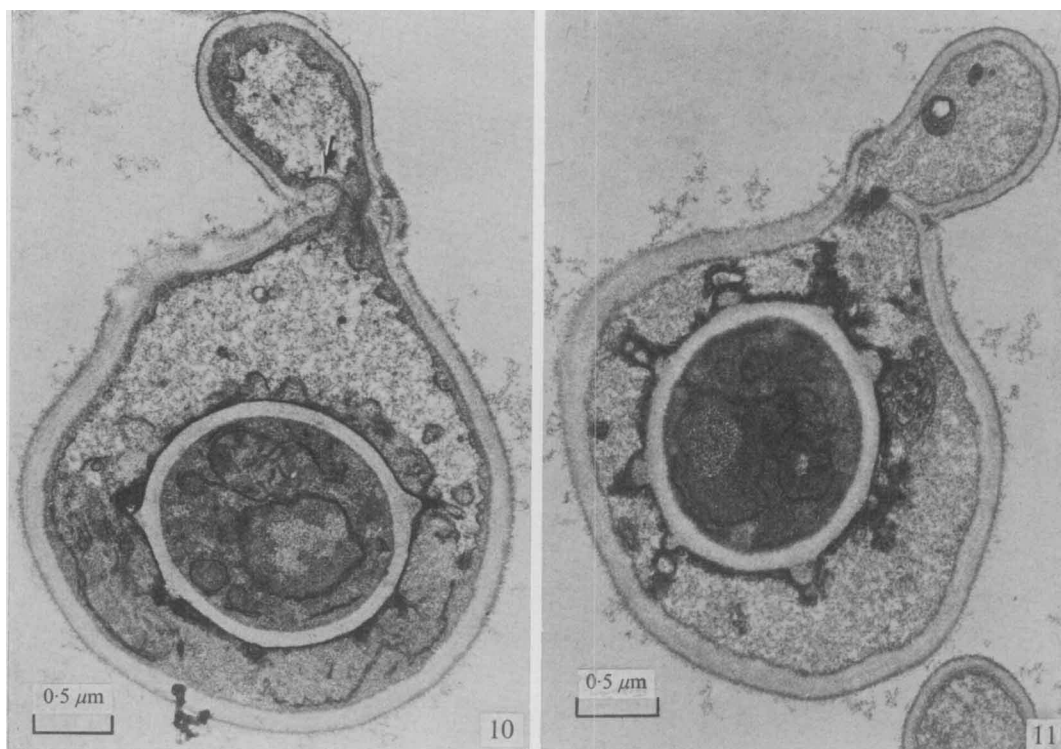


Fig. 10. Ascus with a young spore with small warts. A bulge is visible at the collar between mother cell and bud, and the closure line is present in the remaining part of the cross wall (arrow).

Fig. 11. Ascus with mature spore. The spore wall has distinct warts.

The mature spore did not fill the ascus until germination, at which time it had obviously swollen (Fig. 12). It was occasionally slightly angular and the warts on the wall were flattened. The dark outer layer of the spore wall at first appeared unbroken, though it sometimes appeared to be broken. In the light inner part of the wall two layers were observed separated by an indistinct dark line. The plasmalemma had a crenulated appearance and the organelles of the germinating spore were clearly visible. The swollen spore with the ascus wall still around it formed one or more buds. At the site of budding, the ascus wall was broken or dissolved. The swollen spore budded in the same way as the vegetative cell: the wall of the bud arose from under the wall of the mother cell, which in this case was the wall of the spore (Fig. 13); a cross wall was formed between mother cell and bud, and the bud remained attached to the mother cell or was separated from it. The ascus wall could persist around the spore, or be partly or entirely lost. The preparation examined contained several free germinated spores. These had often lost the dark outer layer of the spore wall and most of the outer part of the light inner layer.



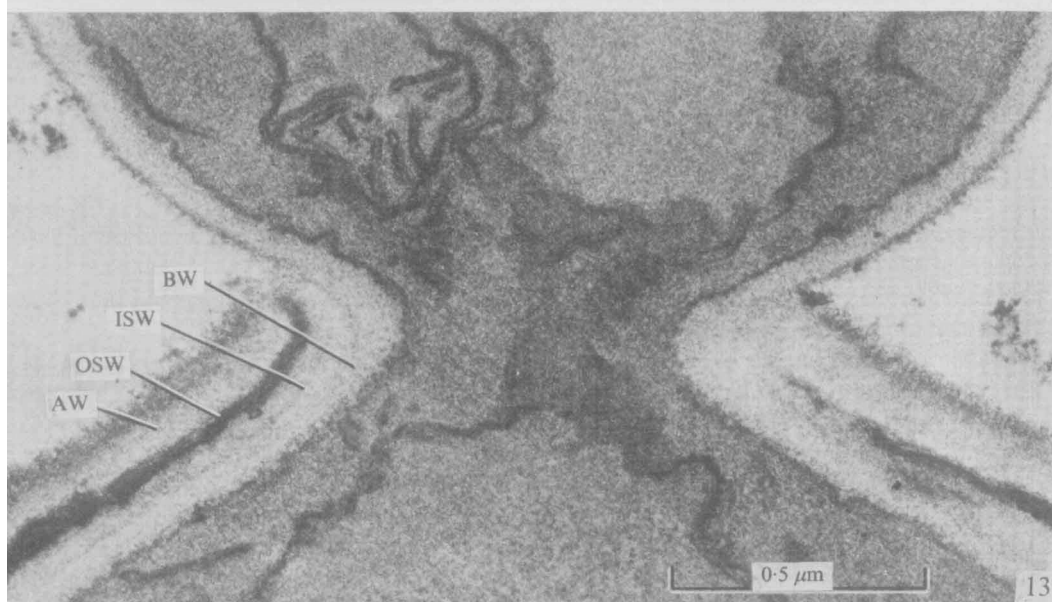


Fig. 12. Ascus with swollen spore which has formed a bud. The bud has penetrated the wall of the ascus and thereby pushed aside the conjugation bud. In the wall of the germinated spore two light layers are separated by an indistinct dark line.

Fig. 13. Section through the collar between mother cell and bud of a germinated spore. The edge of the collar shows several layers: the wall of the ascus (AW), the dark outer layer of the spore wall (OSW), and two light inner layers of the spore wall separated by a dark line (ISW). The wall of the bud (BW) emerges from under these layers.



## DISCUSSION

From our observations it appears that before conjugation between mother cell and bud, the two cells are separated completely by a cross wall. This is in agreement with Guilliermond's (1911) description and unlike that of van der Walt (1970).

The conjugating bud is generally small and often one of several others still attached to the mother cell. This type of bud formation ('Kronenbildung') has been described for other haploid yeasts (Krumbholz, 1931). There is no evidence that the conjugating bud differs from the others, but possibly it is the youngest.

Conjugation was preceded by the formation of short outgrowths on mother cell and bud, which we consider to be conjugation tubes. Thus it resembles conjugation between two separate cells as described for *Hansenula wingei* (Conti & Brock, 1965) and *Saccharomyces cerevisiae* (Osumi, Shimoda & Yanagishima, 1974). The action of wall dissolving enzymes may require a special configuration resembling the penetrating stalks of *Endomycopsis* (*Arthroascus*) *javanensis* (Kreger-van Rij & Veenhuis, 1973), although in *E. javanensis* the action is one-sided and there is no cell fusion. However, our observations of conjugation in *D. hansenii* do not exclude direct dissolution of the cross wall between the two cells involved. Conjugation between unrelated cells, although infrequently observed, was also effected by conjugation tubes, but these were not formed from a scar surface. Budding from a scar surface, leading to the so-called multiple scars, has not been observed in cultures of *D. hansenii* with exclusively vegetative reproduction. It is tempting to consider the buds formed by this method in sporulating cultures as modified conjugation tubes.

Sections of conjugating cells suggest that the nuclei of both cells meet in the open connexion between the cells and fuse, after which the diploid nucleus undergoes meiosis in the mother cell. The lobed nucleus of that stage resembles the meiotic nucleus described for *S. cerevisiae* (Moens, 1971; Guth, Hashimoto & Conti, 1972; Beckett, Illingworth & Rose, 1973). In contrast to the four lobes of the nucleus in that species which become the nuclei of the four spores, only one lobe of the nucleus of *D. hansenii* is enveloped by membranes. We have not attempted a three-dimensional reconstruction of the meiotic nucleus as given by Moens (1971) for *S. cerevisiae* and, therefore, do not know whether more than one lobe develops in *D. hansenii*. In two-spored asci this is expected to be the case.

In sections of the prospore wall a thin dark line was visible which has not been described for *S. cerevisiae*, but is probably similar to that observed in young spores of *Eremascus albus* (Kreger-van Rij, Veenhuis & van der Graaf, 1974). In the wall of mature spores of *D. hansenii* this line could not be traced. The warts on the spores which developed as dark extensions of the light layer of the wall resemble the 'secondary wall' of *Ascodesmis* spores described by Merkus (1973). The lipid bodies surrounding the growing and the mature spore were described by Lynn & Magee (1970); Black & Gorman (1971) mentioned membrane-like structures present in the ascus. We did not find an osmiophilic zone around the spores, as described for *Pichia pijperi* and *Kluyveromyces osmophilus* (Kreger-van Rij, 1969).

At germination, the spores change to the vegetative condition. This includes swelling, thereby extending the wall, and new wall material is probably formed. The two zones in the light part of the wall of a germinating spore, separated by a thin dark line, may represent the original spore wall and a new layer formed within. The latter becomes the wall of the vegetative cell as the other layers disappear.

This investigation was supported by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.). We thank Mr D. Yarrow for corrections of the English text.

## REFERENCES

- BECKETT, A., ILLINGWORTH, R. F. & ROSE, A. H. (1973). Ascospore wall development in *Saccharomyces cerevisiae*. *Journal of Bacteriology* **113**, 1054-1057.
- BLACK, S. H. & GORMAN, C. (1971). The cytology of *Hansenula*. III. Nuclear segregation and envelopment during ascosporeogenesis in *Hansenula wingei*. *Archiv für Mikrobiologie* **79**, 231-248.
- CONTI, S. F. & BROCK, T. D. (1965). Electron microscopy of cell fusion in conjugating *Hansenula wingei*. *Journal of Bacteriology* **90**, 524-533.
- GUILLIERMOND, A. (1911). Sur un exemple de copulation hétérogamique observé chez une levure. *Comptes rendus des séances de la Société de biologie* **70**, 442-444.
- GUILLIERMOND, A. (1928). *Clef dichotomique pour la Détermination des Levures*. Paris: Librairie le François.
- GUTH, E., HASHIMOTO, T. & CONTI, S. F. (1972). Morphogenesis of ascospores in *Saccharomyces cerevisiae*. *Journal of Bacteriology* **109**, 869-880.
- KREGER-VAN RIJ, N. J. W. (1969). Taxonomy and systematics of yeasts. In *The Yeasts*. London and New York: Academic Press.
- KREGER-VAN RIJ, N. J. W. & VEENHUIS, M. (1973). Electron microscopy of some special cell contacts in yeasts. *Journal of Bacteriology* **113**, 350-356.
- KREGER-VAN RIJ, N. J. W., VEENHUIS, M. & LEEMBURG-VAN DER GRAAF, C. A. (1974). Ultrastructure of hyphae and ascospores in the genus *Eremascus* Eidam. *Antonie van Leeuwenhoek* **40**, 533-542.
- KRUMBHOLZ, G. (1931). Untersuchungen über osmophile Sprosspilze. II. *Archiv für Mikrobiologie* **2**, 411-492.
- LYNN, R. R. & MAGEE, P. T. (1970). Development of the spore wall during ascospore formation in *Saccharomyces cerevisiae*. *Journal of Cell Biology* **44**, 688-692.
- MERKUS, E. (1973). Ultrastructure of the ascospore wall in Pezizales (Ascomycetes). I. *Persoonia* **7**, 351-366.
- MOENS, P. B. (1971). Fine structure of ascospore development in the yeast *Saccharomyces cerevisiae*. *Canadian Journal of Microbiology* **17**, 507-510.
- OSUMI, M., SHIMODA, C. & YANAGISHIMA, N. (1974). Mating reaction in *Saccharomyces cerevisiae*. V. Changes in the fine structure during the mating reaction. *Archiv für Mikrobiologie* **97**, 27-38.
- SPURR, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* **26**, 31-43.
- VAN DER WALT, J. P. (1970). Criteria and methods used in classification. In *The Yeasts*. Amsterdam: North-Holland.